

Transgenic Animals

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Abstract

This entry describes each of the four primary methods that have been used to transfer genes into farm animals and the methods used to subsequently evaluate the animals that have resulted from the transfer of genes to prove that the genes are present in the genome, are functioning, and are being transmitted to the next generation.

INTRODUCTION

The genetic composition of all living creatures is continually undergoing alteration by mutation, natural selection, and genetic drift. Beginning at the onset of plant and animal domestication, humans have further manipulated the genetic composition of plant and animals to enhance their health and usefulness to humans by selecting for specific phenotypic traits. Development of recombinant DNA technology has enabled scientists to isolate single genes, analyze and modify their nucleotide structures, make copies of these isolated genes, and insert copies of these genes into the genome of plants and animals. The procedure used to insert these isolated genes is called "gene transfer," an animal that contains the inserted gene or genes is called a "transgenic animal," and the transferred gene is called a "transgene."

The first intentional transfer of a transgene into the genome of an animal was achieved in 1980 in mice.^[1] Gene transfer methodology was subsequently successfully applied to pigs, sheep, and rabbits.^[2] Gene transfer has thus far been used most extensively for basic research on all aspects of biology and genetics, but it has numerous potential applications for genetic improvement of farm animals. Practical applications of gene transfer in livestock production include improved milk production and composition, increased growth rate, improved feed use and carcass composition, increased disease resistance, enhanced reproductive performance, and increased prolificacy. Gene transfer in farm animals has also been investigated extensively for potential to produce human pharmaceutical products, and alteration of cell or tissue characteristics for potential use in organ transplantation in humans.

GENE TRANSFER METHODS

Pronuclear Microinjection

The primary method used to produce transgenic farm mammals has been the direct microinjection of the

transgene into the pronucleus of a zygote (recently fertilized ovum or egg). As in the mouse, pronuclei of rabbit, sheep, and goat zygotes can be readily seen using phase-contrast microscopy or differential interference contrast (DIC) microscopy. Lipid granules in the cytoplasm interfere with visualization of pronuclei of pig and cow zygotes. Centrifugation of pig and cow zygotes can be used to stratify the cytoplasm so that pronuclei are visible with use of DIC microscopy.^[3]

To permit microinjection, ova are placed on a depression slide in a microdrop of media that is overlaid with silicone or paraffin oil to prevent evaporation. The microscope must be equipped with two micromanipulators, one for an egg-holding pipette and the other for an injection pipette. The holding pipette and injection pipette are each fitted with a tube leading to a syringe that permits either gentle suction or carefully controlled fluid injection. As an ovum is held with light suction by the holding pipette, the tip of the injection pipette is inserted through the zona pellucida and cytoplasm into the most visible pronucleus. Several hundred copies of the gene are expelled into the pronucleus. The person performing the injection carefully observes the pronucleus and withdraws the pipette when the pronuclear structure has visibly enlarged. After microinjection, cow embryos are usually cultured *in vitro* until they are morulae or blastocysts before non-surgical transfer into the uterus of a synchronous host cow. The injected zygotes of the other species are usually cultured only a few hours before they are surgically transferred directly into the oviduct of synchronous host females.

The mechanism by which a transgene integrates into a chromosome is unknown. Usually multiple copies of a transgene integrates in a head to tail array in a single site on a chromosome but multiple integrations can occur. If a transgene integrates in a zygote after DNA replication has occurred or at a subsequent stage of development, then the transgenic founder will be genetically mosaic since the transgene will only be present in a portion of the cells. Breeding studies with transgenic pigs and sheep indicate mosaicism is a definite problem. As a consequence, about

20% of transgenic founder animals fail to transmit the transgene to progeny and another 20–30 % transmit the transgene to less than 50 % of their progeny.

The efficiency is usually lower for integration of transgenes into farm animals than into mice. The percentage of gene-injected zygotes that develop into transgenic animals varied from 0.3 to 4.0% for pigs, 0.1 to 4.4% for sheep, 1.0 to 1.7% for goats, and 0.3 to 2.6% for cattle. A few transgenic chickens have been produced by microinjection of genes into the germinal disk of the recently fertilized egg.^[4] After microinjection, the chick embryos were cultured in a host eggshell until hatching time.

Retroviral Insertion

Retroviruses can be modified by recombinant DNA techniques to make them replication-defective and replace part of the viral DNA with a desired transgene so they can then be used as a gene vector. Retroviral-mediated gene transfer was originally used to insert transgenes into mouse embryos^[5] and blastodermal cells of chicken eggs.^[6] In comparison to microinjection, retroviral infection offers advantages of: 1) integration of single copies of the gene; and 2) retroviral DNA integrates into a high percentage of embryos when exposed to high concentrations of viral stock by coculture with infected cells in vitro, or in the case of chickens, by microinjection into the blastodisk. The disadvantages are: 1) added work to produce a retrovirus carrying the transgene; 2) the gene being transferred must be smaller than 10 kb in size; 3) resulting transgenic animals are frequently highly mosaic, which necessitates extensive outbreeding to establish pure transgenic lines; and 4) unresolved problems with hypermethylation interfering with expression of the transgene.

More recently, retrovirus-mediated gene transfer has been used to produce transgenic cattle by insertion of retroviruses into the metaphase II oocytes to avoid mosaicism and ensure that a high percentage of the offspring are transgenic.^[7]

Many laboratories involved in production of transgenic livestock have not embraced the use of retroviral insertion technology because of concerns about public perception and the potential consequences of recombination events between the viral vectors and endogenous retroviruses to generate new pathogenic agents.

Cellular Insertion

The third method of introducing genes into the germ line is a two-step process involving first the transfection of a transgene into embryonic stem (ES) cells, embryonic germ (EG) cells (also known as primordial germ cells), or fetal somatic cells during in vitro culture, and then incorporating the transgenic ES or EG cells into an inner cell mass of an embryo or inserting the transgenic cell's nucleus into an enucleated oocyte by nuclear transfer (NT). The

advantage of this procedure is that a particular genotype can be selected during in vitro culture before introduction of the cells into the embryo or NT. In addition, this technique provides the ability for site-specific insertion of a transgene by homologous recombination. This approach with ES cells has been used extensively in the mouse but has not been effective in other mammalian animals because of extreme difficulty in isolating and maintaining ES or EG cells in the undifferentiated state during in vitro culture. Nuclear transfer (also known as animal cloning) is currently being extensively investigated in cattle, goats, and pigs. Consequently, cellular insertion by NT may become the method of choice for gene transfer in these species because relatively few recipient hosts are required to produce transgenic founder animals.

Sperm-Mediated Gene Transfer

The simplest but most controversial method of gene transfer involves merely mixing a transgene with spermatozoa and using them to fertilize oocytes, either in vitro or by artificial insemination. The use of sperm-mediated transfer in mice by Dr. Lavitrano^[8] was initially discounted as unrepeatable by many investigators. During the past decade, research on this procedure has persisted and many investigators report successful gene transfers by this technique. Only a few studies have provided convincing evidence that the transgene was unaltered before or during the integration process and capable of expressing appropriately in the resulting transgenic animals.^[9] In addition, recent investigations indicate that most of genes transferred by this method are not integrated into the host genome but remain extrachromosomal and replicate independent from the host genome.^[10]

EVALUATION OF TRANSGENIC ANIMALS

Integration

The primary way that the presence of the transgene is confirmed is by removing a piece of tail tissue at birth, extracting the DNA from the tissue, and analysis of the DNA for the presence of the transgene by Southern blot hybridization, slot-blot hybridization, or polymerase chain reaction using a unique segment of the transgene as a probe. If performed correctly, the Southern blot analysis provides information on presence, intactness, copy number, and orientation of the transgene.

Expression

Mere presence of the transgene in the transgenic animal does not guarantee that the transgene will be expressed or expressed appropriately. Expression is usually evaluated by assay of appropriate tissues or fluids recovered from the

transgenic animal for presence of the transgene transcription by Northern blot analysis of the mRNA, by presence of the protein using Western immunoblot analysis, or by some other assay that is appropriate for specific transgene.

Transmission

The primary aim of transgenesis is to establish a new genetic line of animals in which the trait is stably transmitted to succeeding generations. This can only be determined by mating the transgenic animal to a non-transgenic animal and subsequent evaluation of the progeny for presence and expression of the transgene.

CONCLUSION

Transgenic animals were initially produced by pronuclear microinjection. During the past two decades, a number of other gene transfer methods have been developed and include use of retroviral vectors, cellular insertion, and sperm-mediated transfer. Additional details regarding each of these procedures can be found in *Transgenic Animal Technology: A Laboratory Handbook*.^[11]

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